REMARKS

Claims 1-43 are pending. Claims 1-3 are examined and rejected while the remaining claims are withdrawn from consideration. Purely in the interest of advancing prosecution, Applicants herein amend claim 1 to recite that the claimed ligands are diagnostic and that they bind to the hypusine-containing form of eukaryotic initiation factor 5A. No issue of new matter arises as express support for these two recitations may be found throughout the specification, such as, for instance, in paragraph [0012].

Rejection under 35 U.S.C. 102

The Examiner rejects claims 1-3 drawn to "a ligand binding to the hypusine region of eukaryotic initiation factor 5A" as allegedly taught by the prior art as follows:

1. Bergeron *et al., J. Med. Chem.*, 1998; 41(20):3888-3900 allegedly teach monoclonal antibodies generated to amino acid residues 48-52 of SEQ ID NOs: 1 and 2;
2. Ruhl *et al., J Cell Biol.* 1993; 123:1312-1320 allegedly teach an antibody binding to elF-5A, allegedly the same as SEQ ID NO:2;
3. Beninati *et al., FEBS Letters* 437:34-38 allegedly teach an antibody binding to hypusine containing elF-5A; and
4. Xu *et al., J Biol Chem* 2001; 276:2555-2561 allegedly teach a ligand binding to hypusine containing elF-5A.

Applicants respectfully traverse. Further, Applicants amend the claims to further distinguish the claimed ligands from the prior art. First, they are now described as "diagnostic." Second, they are now described as interacting with a hypusine-containing form of eukaryotic intiation factor 5A.

Claims 1-3 do not embrace a diagnostic ligand, such as an antibody, that interacts with eIF5A in general. Rather, the present claims embrace a diagnostic ligand that interacts with a *hypusine-containing* eIF5A, i.e., with just the post-translationally hydroxylated form of the eIF5A protein. This form is distinct as a result of the enzymatic incorporation of a single oxygen atom into the modified lysine ('deoxyhypusine') residue, occurring at position 50 in human eIF5A. A ligand interacting with just a subpopulation of eIF5A is distinct from the prior art. This finding, i.e. the reactivity of an antibody stringently recognizing a 0.094 %

difference in molecular weight brought about by the post-translational hydroxylation of eIF5A, is a central feature of the present invention.

1. **Regarding Bergeron *et al., J. Med. Chem.*, 1998; 41(20):3888-3900**

Bergeron *et al., Journal of Medicinal Chemistry* 1998; 41:3888-3900 do not teach or suggest a ligand according to claims 1-3. Table 2 of Bergeron *et al.* presents the reactivity of antibodies merely against the *synthetic peptides* used to raise the same. Table 2 does not show the reactivity of antibodies against a naturally occurring, unhydroxylated or hydroxylated eIF5A protein or parent polypeptide. In fact, Bergeron *et al.* teach that synthetic peptides precisely modeled on the unhydroxylated or hydroxylated sequence around position 50 of human eIF5A do not produce antibodies reactive with naturally occurring eIF5A, regardless of hydroxylation. In the last paragraph of the section entitled 'Hypusine monoclonal antibody generation,' Bergeron *et al.* concede as follows:

> "It is important to point out that ***none of the antibodies cross-reacted with the eIF-5A parent polypeptides*** as tested by ELISA against ***recombinant human eIF-5A precursor polypeptide in which Lys-50 had not been posttranslationally modified*** and against ***a protein fraction from CHO cells known to contain hypusinated eIF-5A***. ... the isolated antibodies do **not** cross-react with these constitutively expressed cellular proteins..." (p. 3893; emphasis added)

As such, Bergeron *et al.* teach that sequence-related epitopes are insufficient for generating recognition of human eIF5A by an immunologic ligand. The presently pending claims are directed to ligands that precisely recognize only the hydroxylated form of eIF5A. Bergeron *et al.* do not teach or suggest such ligands.

2. **Regarding Ruhl *et al., J Cell Biol.* 1993; 123:1312-1320**

Ruhl *et al., Journal of Cell Biology* 1993; 123:1312-1320 do not teach or suggest the presently claimed ligands. The Examiner says that antibodies reactive to eIF5A have been known in the art for many years before the filing date of the present application. However, Applicants do not claim any reagent reactive with eIF5A in general. Rather, their claims

presently pending are directed to diagnostic ligands that specifically interact with a *hypusine-containing* eIF5A *only*.

Ruhl *et al.* do not teach the hydroxylation status of the eIF5A against which their antibodies react. (*See*, Table 1) The terms 'hydroxylated' or 'unhydroxylated' or 'hydroxylation' are absent from the reference. Ruhl *et al.* mention the term 'hypusine' only once and then only cursorily. Ruhl *et al.* do not teach or suggest that the hydroxylation of naturally occurring eIF5A might be exploited to generate a hydroxylation-specific signal. Of note, the antibodies Ruhl *et al.* generated produce a staining pattern in non-transfected cells that differs completely from the staining pattern established by the reagents according to the present invention. The reagent of Ruhl *et al.* generates a prominent nuclear signal (Fig. 7), whereas Applicants' reagent fails to stain the nucleus. (*See*, Cracchiolo *et al., Gynecologic Oncology* 2004: 94:217 – 222, submitted herewith as Exhibit A, high power inset into Fig. 1A).

3. Regarding Beninati *et al., FEBS Letters* 437:34-38

Beninati *et al.* do not teach or suggest the hydroxylation status of the eIF5A against which their antibodies react. Beninati *et al.* merely juxtapose, in Fig. 1, metabolic labeling for hypusine formation (Fig. 1A) and immunologic labeling for eIF5A synthesis (Fig. 1B/C). The color scheme in Fig. 1A is the reverse of the color scheme in Fig. 1C. Remarkably, Beninati *et al.* do not find congruency between these measurements, i.e. the degree of eIF5A synthesis and of eIF5A hydroxylation. Specifically, in the last paragraph of section 3.1 of their results, Beninati *et al.* concede the dissociation of these parameters:

> "The data showed an about 30% decrease of the intensity of the eIF5A band [***Comment: as measured via Western with their anti-eIF5A antibody***] ... that did **not** account for corresponding changes in hypusine synthesis [***Comment: as measured with metabolic labeling***]" (p. 36; bolded for emphasis)

As such, Beninati *et al.* do not teach or suggest a ligand that binds to eIF5A in a manner dependent on eIF5A hydroxylation. Beninati *et al.* establish that their anti-eIF5A antibody fails to capture eIF5A hydroxylation. Therefore, Beninati *et al.* do not teach or suggest a ligand having reactivity against eIF5A *dependent on just the hydroxylation of the* eIF5A as presently claimed.

4. Regarding Xu *et al., Journal of Biological Chemistry 2001; 276: 2555-2561*

Xu *et al., Journal of Biological Chemistry* 2001; 276:2555-2561 do not teach or suggest the diagnostic ligands presently claimed . The intention of Applicants is not to claim all ligands that bind to hydroxylated eIF5A, but to claim ligands useful for diagnosis of human diseases dependent on hydroxylated eIF5A, and for detection of the tissue response to therapeutics that inhibit synthesis of hydroxylated eIF5A. Such diseases include cancer and its obligate precursor, intraepithelial neoplasia. (*See*, Cracchiolo *et al., Gynecologic Oncology* 2004: 94:217 – 222, submitted herewith as Exhibit A) The therapeutics include, for instance, the drugs ciclopirox and deferiprone. (*See*, Hoque *et al., Retrovirology* 2009; 6:90-107, submitted herewith as Exhibit B). Exposure of eIF5A to random-sequence RNA ligands may be used in sequential cycles to enhance those RNA ligands that bind only to hydroxylated eIF5A, as taught by Xu *et al.* However, RNA ligands are known to those experienced in the art as not useful for the diagnostic identification of biomarkers in biological samples, since RNA of whatever sequence is, in contrast to DNA, exquisitely susceptible to degradation by ubiquitous RNA-digesting enzymes (RNAses). In fact, Applicants are not aware of a single diagnostic method that relies on an RNA ligand to probe a biological sample for a diagnostic biomarker signal. Of note, Xu *et al.* used the sequential cycle enhancement of random-sequence RNA ligands to identify those mRNA species whose translation at the ribosome might be controlled by hydroxylated eIF5A. However, the present claims recite 'diagnostic' to describe the 'ligand' claimed. 'Diagnostic ligand' excludes RNA due to biological instability of RNA.

5. Further data demonstrating the patentability of the presently claimed ligands

Applicants have established in further detail that the presently claimed ligands recognize eIF5A *dependent on just the hydroxylation of the latter.* Applicants refer to Exhibit B, Hogue *et al., Retrovirology* 2009; 6:90-107. Applicants show that NIH-353 provides a dose-dependent decrease in immune reactivity towards cellular and transfected FLAG-labeled eIF5A in the presence of several established inhibitors of eIF5A

hydroxylation. Of note, the FLAG antigenicity is not reduced by these drugs, as demonstrated in Fig. 2C, only the 'hydroxylation-generated' antigenicity is reduced per the reagent. This finding occurs in strict congruency with metabolic labeling for hypusine formation, which Applicants published earlier (e.g., *International Journal of Cancer* 2002; 100:491–498). Thus, Applicants' ligands detect a pharmacological reduction in hydroxylated eIF5A even if huge amounts of unhydroxylated eIF5A are present in the same cells.

Applicants submit that the simple citation of the human peptide sequence around the lysine-derived hydroxylated hypusine residue of eIF5A, as provided by Bergeron *et al.* (page 3888) and by Ruhl *et al.* (in Fig. 4) is not evidence that Bergeron *et al.* and Ruhl *et al.* teach reactivity of their antibodies with just hydroxylated eIF5A. Likewise, Applicants submit that neither Bergeron *et al.* nor Ruhl *et al.* teach or suggest selective reactivity of a ligand with hydroxylated eIF5A would be possible. In fact, Ruhl *et al.* cite the sequence around the unique hypusine region of eIF5A for the sole purpose of establishing the identity of a cellular factor noted to bind Rev of HIV-1. Bergeron *et al.* do test selective reactivity, but reject it for naturally occurring, endogenous eIF5A, whether hydroxylated or not and acknowledge reactivity only for "tracking the localization of microinjected [*Comment: thus exogenous*]...peptides." (*See*, p.3893, emphasis added). The reference to the sequence around the hypusine residue and the term 'hypusine region' can therefore become confounders. Accordingly, Applicants herein amend claim 1, substituting the term '*binding to the hypusine region of eukaryotic initiation factor 5A*' with the term '*binding to the hypusine-containing form of eukaryotic initiation factor 5A*'

To highlight Applicants' unique and unanticipated discovery of hydroxylation-specific anti-eIF5A antibodies, neither taught nor suggested by Bergeron *et al.*, Ruhl *et al.*, Beninati *et al.*, nor Xu *et al.*, as set forth above, Applicants submit the following Table for explanation:

Table 1.

	eIF5A reactive material	Lys-50 form Unhydroxylated eIF5A	Hpu-50 form Hydroxylated eIF5A	Staining of human tissues for diagnostics
Applicants (NIH-353) Refs. 1-7	Antibody	(-)	(+++)	No nuclear stain, only cytoplasmic stain
Bergeron *et al.* Ref. 8	Antibody	(-)	(-)	N/A
Ruhl *et al.* Ref. 9	Antibody	(?)	(?)	Only nuclear stain, no cytoplasmic stain
Beninati *et al.* Ref. 10	Antibody	(?)	(?)	(?)
Becton & Dickinson Ref. 11	Antibody	(+++)	(+++)	Nuclear stain and cytoplasmic stain
Xu *et al.* Ref. 12	RNA	(-)	(+)	N/A

Lys, lysine (in unhydroxylated eIF5A); Hpu, hypusine (in hydroxylated eIF5A); N/A, not applicable

(-) non-reactive; (+) reactive; (?) not specified, no evidence, not taught.

Applicants include the anti-eIF5A reagent commercially distributed by Becton-Dickinson (San Jose, CA) under BD Biosciences Material Number 611977. This antibody was generated against a recombinant form of human eIF5A in which the entire hypusine-containing part of the molecule was intentionally deleted so as to avoid interference with Applicants' pending patent application. As expected, this antibody does not distinguish between hydroxylated and unhydroxylated eIF5A, as established by Applicants in ref. 1, Fig. 1, and in human tissues displays both nuclear and cytoplasmic staining, as observed by Applicants.

References of Table 1.

1. Cracchiolo *et al., Gynecologic Oncology* 2004; 94:217 – 222
2. Hoque *et al., Retrovirology* 2009; 6:90-107
3. Heller *et al.,* (2003) Eukaryotic Translation Initiation Factor 5A, An Emerging Target For Cytostatic Compounds, Localizes To Cervical Carcinomas. Biennial Meeting of the American Society for Colposcopy and Cervical Pathology, Orland / Florida. J. Lower Gen. Tract. Dis. 8, 234.
4. Hanauske-Abel *et al.,* (2004) The mature, hypusine-containing form of eukaryotic translation initiation factor 5a (EIF5A) in normal ovaries and in ovarian cancer: anatomical evidence for a potential biomarker. 51st Annual Meeting of the Society for Gynecological Investigation, Houston / Texas. J. Soc. Gynecol. Invest. 11, 404A (#975);
5. Cracchiolo *et al.,* (2004) Hypusine-containing eIF-5A in biopsies of genital cancers : Evidence for a pivotal role of translational control in malignancies. 35th Annual Meeting of the Society of Gynecologic Oncologists, San Diego / California.

6. Heller, *et al.,* (2003) Eukaryotic Translation Initiation Factor 5A(eIF-5A) Localizes to Vulvar Intraepithelial Neoplasia. Conference of the International Society for the Study of Vulvovaginal Diseases (ISSVD), Salvador/Brazil.
7. Heller, *et al.,* (2004) Expression of eIF5A is greater in type II than in type I uterine cancers. Conference of the United States and Canadian Academy of Pathology, Vancouver / Canada. Modern Pathology, 17 (supp. 1): 828A
8. Bergeron *et al., J. Med. Chem.*, 1998; 41:3888-3900
9. Ruhl *et al., Journal of Cell Biology* 1993; 123:1309-1320
10. Beninati *et al., FEBS Letters* 1998; 437:34-38
11. BD Transduction Laboratories™, Technical Data Sheet Bioimaging Certified Reagent; Purified Mouse Anti-eIF-5a [http://www.bdbiosciences.com/external_files/pm/doc/tds/tl/live/web_enabled/E15920_611977.pdf#search=(Anti-eIF5A)]
12. Xu *et al., Journal of Biological Chemistry* 276:2555–2561.

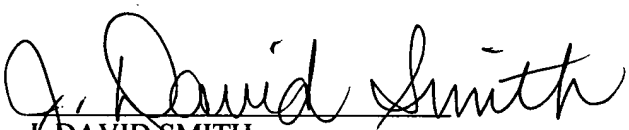
FEES

No additional fees are believed necessary in connection with the present submission; however, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment or to credit any overage.

CONCLUSION

It is believed that all of the claims are patentable and early notification as such is earnestly solicited. If any issues may be resolved by way of telephone, the Examiner is invited to call the undersigned at the telephone number indicated below.

Respectfully submitted,



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